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| (51) International Patent Classification ⁶: G01N 33/558, 33/543, 33/577, 33/58 | A1 | (11) International Publication Number: WO 97/27484 (43) International Publication Date: 31 July 1997 (31.07.97) |
| (21) International Application Number: PCT/GB97/00223 (22) International Filing Date: 24 January 1997 (24.01.97) (30) Priority Data: 9601404.8 24 January 1996 (24.01.96) GB (71) Applicant (for all designated States except US): INDOOR BIOTECHNOLOGIES LIMITED [GB/GB]; 123 Deansgate, Manchester M3 2BU (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): CHAPMAN, Martin, Dudley [GB/US]; 1717 King Mountain Road, Charlottesville, VA 22901 (US). (74) Agents: O'BRIEN, Caroline, J. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB). | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> | |
| (54) Title: ALLERGEN DETECTION ASSAY (57) Abstract <p>The invention provides a method for testing for the presence of a particular allergen which comprises (a) obtaining a substantially unpurified sample suspected of containing an allergen; (b) if a non-liquid sample mixing it with a liquid diluent and if a liquid sample optionally diluting it with liquid diluent to produce a liquid sample being a solution and/or suspension of the sample; (c) contacting the liquid sample or a portion thereof with a labelled first binding reagent for a said allergen which reagent is freely movable through a porous support of an assay device; (d) allowing the first binding reagent whether free or complexed to allergen to move along a said porous support to meet a second binding reagent for a said allergen immobilised at a detection zone in the support, wherein the first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a said binding reagent is specific for an epitope characteristic of said allergen; and (e) examining for labelled binding reagent at the detection zone, the presence of labelled binding reagent at the detection zone being indicative of the absence/presence of allergen in the sample, depending on whether the first and second binding reagents are with respect to each other, competitive or non-competitive for said allergen.</p> | | |

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ALLERGEN DETECTION ASSAY

5 The present invention relates to an assay for detecting the presence of environmental allergens in eg foods, air, dust such as house dust, and in particular to a one-step rapid assay of the "dipstick" type.

BACKGROUND OF INVENTION

10 Allergens present in the environment (eg indoor allergens such as occur in the home) are a major risk factor in the onset of allergic diseases or in exacerbating the symptoms of such disease. (T.A.E.Platts-Mills, Annals of Allergy, (1994), 72, 4, 381-384). Particular examples of
15 such diseases are rhinitis, asthma, hayfever or eczema.

Patients may suffer an allergic response if exposed to allergens such as plant or animal allergens present in eg food, dust etc. Examples of the most common domestic
20 allergens which are implicated in allergic diseases include, for example, those derived from house dust mites or insects such as cockroaches and flies, domestic pets such as cats and dogs, and plant allergens such as pollen, as well as fungal allergens. Other allergens may
25 be presented by foodstuffs such as nuts, or industrial substances such as latex.

Sensitivity to allergens is an increasing problem. In some cases, it appears that patients have a genetic
30 predisposition to develop allergies following exposure to allergens in their environment. Some patients become sensitised to particular allergens which are present at high levels in their environment. For instance, in regions of the world where house dust mites are
35 prevalent, it is found that a large number of patients suffering from asthma are allergic to house dust mite allergen. However, in other areas, for example where the

humidity and conditions such as at high altitudes are not conducive to house dust mites, e.g. in the Alps or in Los Alamos, New Mexico, USA, or in arid desert regions, asthma caused by other types of allergen such as those produced by household pets such as cats or dogs, may be a more prevalent cause of allergic disease (Sporik et al., Am J Respir Crit Care Med, 151, (1995) pp1388-1392).

PRIOR ART

Diagnostic tests are available which enable a doctor to pinpoint with a high degree of accuracy, the particular allergens which give rise to an allergic response in a particular patient. An example of such a test is the so called "skin-prick" test where a range of allergens are introduced into the surface of the skin along a patients arm, and the reaction to each of these observed.

Once diagnosed, symptoms of the disease can be alleviated or even prevented by taking appropriate preventative measures eg by ensuring that the patient is not exposed to unacceptably high levels of that particular allergen, especially in their home environment. In the case of allergy to house dust mites, one can consider using specially designed bed covers which isolate the patient from their mattress which is a particular source of such allergens. Alternatively, chemical treatments are available which would eliminate or reduce the mites themselves, or chemically destroy the protein allergens. In order to assess the appropriate treatment strategy, exposure to allergens and in particular indoor allergens has to be monitored (M. D. Chapman et al., Allergy 1995, 50, 29-33).

Tests are available for testing the presence of allergens in house dust or even in air. The dust is first collected, typically using the sort of device and the sampling strategy described by Tovey E.R. et al., American

Review of Respiratory Disease 1981: 124:630-636, or
T.A.E. Platts-Mills et al, Allergy and Clinical
Immunology 1992: 89: No 5, pp1046-1060. The devices used
include moulded plastic attachments for fitting on the
wand of a vacuum cleaner and which contain a membrane of
paper filter, inside upon which dust is collected (eg as
available from ALK Laboratories, Horsholm, Denmark). A
further device, available from Allergy Control Products,
Ridgefield, Connecticut, USA comprises a fiber bag-like
receptacle with a plastic rim portion, which fits on the
end of the vacuum cleaner so that the bag fits inside the
pipe. When the vacuum cleaner is used, dust collects in
the bag and can then be extracted.

Collected dust is then sent to laboratories where
allergens are extracted and they are tested for using the
microplate based enzyme-linked immunoabsorbent assay
(ELISA) tests. Typically, in these tests, antibodies are
absorbed onto a plastic surface, allergen extract is
added, and bound allergen detected using a labelled
second antibody. The second antibody is detected using a
ligand coupled to an enzyme which can be visualised by
addition of a chromogen. However this technology is
difficult to adapt for home use and cannot be used by
patients themselves. A review of immunoassays for indoor
allergens can be found in Chapter 11 of "Bioaerosols"
Ed. Harriet A. Burge, (1995), CRC Press Inc..

Another type of test available specifically for house
dust mites is the so-called "guanine test" (Bischoff E et
al., Allergologie 1984: 11: 446-449, JEM Van Bronswijk et
al., J. Medical Entomology 1989: 26: 55-59). In this
test, house dust samples are tested chemically for the
presence of guanine which is indicative of the presence
of house dust mites using a dipstick type test. The
results obtainable using this test are not altogether
satisfactory (M. D. Chapman, Allergy, 1993, 48: 301-302)

as the sensitivity levels achievable are not very high. Furthermore, it can be used only to detect the presence of one source of allergens, house dust mites, and so is of no use where such allergens are not implicated in a particular allergy.

EP-A2-0615,128 discloses a dipstick based test in which a dipstick carries polyclonal anti-mite antibodies. To this is then added a sample of house dust in suspension in a solution of more polyclonal anti-mite antibody, but this time conjugated to a dye. The teaching in this document is the use of a polyclonal antiserum which comprises a range of antibodies with differing specificities and affinities for mites.

EP-A1-0498,124 relates to a more specific assay which utilises monoclonal antibodies. The antibodies are used in microtitre wells to which house dust extract is added.

Shattock, A.G. et al in J. Allergy Clin. Immunol. January 1994 "A novel latex agglutination assay for measurement of Der p1 allergen in house dust samples" describes a slide based assay in which antibody coated latex particles are added to a dust sample on a slide.

The present applicants however, suggest that there remains a need for rapid tests, which can be used to reliably and specifically test for a range of particular allergens quickly and easily and preferably in the local environment such as the home. The development of good rapid test assays for allergens would facilitate better monitoring of allergen exposure in eg the home and workplace and assessment of the efficacy of allergen control procedures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a dipstick for use in the assay: (a) shows the device prior to use and (b) shows the device after use with a typical positive result;

Figures 2, 3 and 4 show a series of developed dipsticks used in assays for Fel d 1 in house dust obtained from homes in UK, USA and Brazil;

Figures 5, 6, 7 and 8 show a series of developed dipsticks used in assays for mite Group 2 allergens Der p 2 and Der f 2 in house dust obtained from different locations in homes in Brazil.

The present invention provides an assay suitably carried out as a one-step "dipstick" type assay. Thus the invention provides an assay for detecting the presence of allergens in a sample (such as dust), which comprises use of a dipstick and reagents which are capable of specifically binding to and detecting a particular allergen which may be present in a sample (such as dust).

DESCRIPTION OF THE INVENTION

According to the present invention there is provided a method for testing for the presence of a particular allergen which comprises: (a) obtaining a substantially unpurified sample suspected of containing an allergen; (b) if a non-liquid sample mixing it or a portion thereof with a liquid diluent and if a liquid sample optionally diluting it or a portion thereof with liquid diluent to produce a liquid sample being a solution and/or suspension; (c) contacting the liquid sample or a portion thereof with a labelled first binding reagent for a said allergen, the reagent being freely movable through a porous support of an assay device; (d) allowing the first binding reagent whether free or complexed to allergen to move along a said porous support to meet a second binding

reagent for a said allergen immobilised at a detection zone in the support, wherein the first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a
5 said binding reagent is specific for an epitope characteristic of said allergen; and (e) examining for labelled binding reagent at the detection zone, the presence of labelled binding reagent at the detection zone being indicative of the absence/presence of allergen
10 in the sample, depending on whether the first and second binding reagents are with respect to each other, competitive or non-competitive for said allergen.

The step of contacting the liquid sample or a portion
15 thereof with labelled first binding reagent may occur in the porous support.

The step of contacting the liquid sample or a portion thereof with labelled first binding reagent may occur in
20 a vessel external to the support.

The first and second binding reagents may with respect to each other, be non-competitive for said allergen.

25 The first and second binding reagents may with respect to each other, be competitive for said allergen.

A binding reagent may comprise a substance with an antibody binding domain with specificity for said
30 allergen.

The antibody binding domain may derive from a monoclonal antibody.

35 A binding reagent may comprise a monoclonal antibody or a fragment thereof.

The first and second binding reagents may both comprise substances with a monoclonal antibody binding domain with specificity for said allergen.

5 The first and second binding reagents may both comprise either a monoclonal antibody or a monoclonal antibody fragment.

10 The first and second binding reagents may both comprise either a monoclonal antibody or a monoclonal antibody fragment, which antibody or fragment has an antibody binding domain with specificity a said epitope characteristic of said allergen.

15 The allergen may be one of cat, cockroach, aspergillus or dust mite.

The method may be for testing for the presence of mite Group 1 allergens, or mite Group 2 allergens or both mite Group 1/Group 2 allergens.

20 The porous support may comprise a nitrocellulose membrane.

25 The porous support may be accommodated within a housing.

The labelled first binding reagent may comprise a gold label.

30 The absence/presence of allergen in the sample may be determinable within 20 minutes of contacting the liquid sample with said labelled first binding reagent. The absence/presence of allergen in the sample may be determinable within 15 minutes. The absence/presence of allergen in the sample may be determinable within 10 minutes. The absence/presence of allergen in the sample may be determinable within 5 minutes.

The method may be used for testing for the presence of a plurality of particular allergens which may be found in the sample.

5 The present invention also provides the use of a method as above for testing for the presence of a plurality of particular allergens which may be found in the sample.

10 The method may utilise a plurality of porous supports, each support being designated for testing for the presence of one particular allergen of the plurality of particular allergens.

15 The method may utilise a single porous support for testing for the presence of more than one particular allergen of the plurality of particular allergens.

20 The method may comprise a plurality of pairs of first and second binding reagents, each pair being for testing for the presence of one particular allergen of the plurality of particular allergens.

25 A common first or second binding reagent may be employed for testing for the presence of more than one particular allergen of the plurality of particular allergens.

The sample may be of dust, water, air, a foodstuff or a drink.

30 Dust may be from any origin. It may be house dust.

Also provided are assay devices for testing for the presence of a particular allergen which may be found in a substantially unpurified sample suspected of containing an allergen said devices comprising (a) a porous support,
35 (b) a labelled first binding reagent which is specific for an allergen found in the sample and which is freely

moveable through said support, and (c), a second binding reagent which specifically binds either said allergen in a manner complementary to that of the first binding reagent or which competes with said allergen for binding to said first binding reagent said second binding reagent being immobilised at a detection site on the support.

Also provided are assay systems for testing for the presence of a particular allergen which may be found in a substantially unpurified sample suspected of containing an allergen which comprises (a) a porous support, (b) a labelled first binding reagent for a said allergen, the reagent being freely movable through a porous support of an assay device, (c) a second binding reagent for said allergen which second reagent is immobilised at a detection zone in the support and wherein the first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a said binding reagent is specific for an epitope characteristic of said allergen.

The porous support may bear a solution or suspension of the substantially unpurified sample suspected of containing an allergen.

The labelled first binding reagent may be in a vessel external to the support.

The labelled first binding reagent may be in the porous support.

The second binding reagent may specifically bind said allergen in a manner complementary to that of the first binding reagent.

The first and second binding reagents may be with respect to each other non-competitive for said allergen.

The first and second binding reagents may be with respect to each other competitive for said allergen.

5 A binding reagent may comprise a substance with an antibody binding domain with specificity for said allergen.

10 The antibody binding domain may derive from a monoclonal antibody.

A binding reagent may comprise a monoclonal antibody or a fragment thereof.

15 The first and second binding reagents may both comprise substances with a monoclonal antibody binding domain with specificity for said allergen.

20 The first and second binding reagents may both comprise either a monoclonal antibody or a monoclonal antibody fragment.

25 The first and second binding reagents may both comprise either a monoclonal antibody or a monoclonal antibody fragment, which antibody or antibody fragment has an antibody binding domain with specificity a said epitope characteristic of said allergen.

30 The allergen may be one of cat (such as Fel d 1), cockroach (such as Bla g 2), aspergillus or dust mite (Group 1 and 2).

35 The assay system may be for testing for the presence of mite Group 1 allergens, or mite Group 2 allergens or both mite Group 1/Group 2 allergens.

The porous support may comprise a nitrocellulose membrane.

The porous support may be accommodated within a housing.

The labelled first binding reagent may comprise a gold label.

5

The absence/presence of allergen in the sample may be determinable within 20 minutes of contacting the liquid sample with said labelled first binding reagent. The absence/presence of allergen in the sample may be determinable within 15 minutes. The absence/presence of allergen in the sample may be determinable within 10 minutes. The absence/presence of allergen in the sample may be determinable within 5 minutes.

10

15

Also provided are assay systems as above for testing for the presence of a plurality of particular allergens which may be found in the sample.

20

Such an assay system may utilise a plurality of porous supports, each support being for testing for the presence of one particular allergen of the plurality of particular allergens.

25

Alternatively, such an assay system may utilise a single porous support for testing for the presence of more than one particular allergen of the plurality of particular allergens.

30

An assay system for testing for the presence of a plurality of particular allergens which may be found in the sample may comprise a plurality of pairs of first and second binding reagents, each pair being for testing for the presence of one particular allergen of the plurality of particular allergens.

35

A common first or second binding reagent may be employed for testing for the presence of more than one particular

allergen of the plurality of particular allergens.

The sample may be of dust, water, air, a foodstuff or a drink.

5

Dust may be from any source. It may be house dust.

10

Also provided is an assay device for testing for the presence of a plurality of particular allergens which may be found in a substantially unpurified sample suspected of containing a plurality of allergens said device comprising pairs of binding reagents specific for separate allergens of the plurality, each pair of binding reagents comprising a said first labelled binding reagent (b) and a said second binding reagent (c).

15

20

Also provided is a kit for detecting allergens which comprises a plurality of assay devices as above, each one being capable of detecting a different allergen. The sample may be as stated above.

25

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35

As stated above, the labelled first binding reagent may be added to the liquid sample prior to administration to the support. Alternatively the labelled first binding reagent may be present at site on the support so that it comes into contact with the liquid sample as it moves along the support and is transported with it towards the detection zone. Initially the labelled first binding reagent will bind to allergen present in the sample. On reaching the detection zone, if the second binding reagent is non-competitive with said first binding reagent for said allergen the allergen/labelled first binding reagent complex will bind and become fixed in the detection zone, the allergen being sandwiched between said second binding reagent and said labelled first binding reagent (a sandwich type assay). The label produces a detectable signal which may be observed

depending on the precise nature of the label, either directly, or by use of some signal developing reagent.

Alternatively, if the second binding reagent competes with said allergen for binding to said first binding reagent, the complex will not be fixed in the detection zone and the absence or relative absence of a signal from said label indicates that the sample contains allergen (a competitive assay).

Preferably, for home use, the assay device is arranged as a sandwich type assay.

In a preferred embodiment, the assay is sufficiently sensitive to allow detection of allergens present in a extract formed by mixing a sample such as dust with a diluent such as water or buffered saline. The level of sensitivity is suitably such that it will detect allergens which are present in concentrations which are high enough to be clinically significant in that they are likely to have an affect on allergic patients. This will generally mean that the assay should detect the presence of allergens at levels of less than 50 ng, suitably less than 5 ng and sometimes less than 300 pg although this will depend to a certain extent on the nature of the samples, the allergen being detected, extraction volumes and conditions. Assays exemplified herein show sensitivities of around 100 pg.

Suitably at least one of the first and second binding reagents comprises a substance with an antibody binding domain eg antibodies or binding fragments thereof which retain specificity for said allergen. Antibodies may be polyclonal. Preferably at least one of the first and second binding reagents comprises a substance with an antibody binding domain of a monoclonal antibody. Antibodies to specific allergen may be generated using

conventional techniques. Typically natural allergens purified in accordance with conventional biochemical techniques will be used to raise antibodies. However, the DNA of many clinically significant allergens has been cloned and sequenced (e.g EP-A-362290, International Patent Application No. PCT/US93/08518, International Application No. PCT/AU94/00117, International Application No. PCT/94/00292, International Application No. PCT/US94/14073 and International Application No. PCT/CA93/0050). This therefore also allows the generation of pure recombinant allergen which may be used to generate antibody in a mammal such as a mouse (see for example I.G. Ovsyannikova et al. J. Allergy Clin Immunology, 94, 3 Part 1 pp537-546).

Suitable antibodies for use are available in the art. Particular examples of antibodies which may be used include antibodies which are specific for Group 1 and 2 dust mite allergens, cat allergens such as Fel d 1, and cockroach allergens such as Bla g 2. Suitable antibodies include those set out in the following Table 1.

Table 1

Allergen

Antibody

| | |
|---|--|
| <i>D. Pteronyssinus</i> Der p 1 | mAb 5H8 Biotinylated mAb 4C1 mAb 10B9 Biotinylated 5H8 mAb 4C1 |
| <i>D. farinae</i> Der f 1 | mAb 6A8 Biotinylated mAb 4C1 |
| <i>Dermatophagoides</i> spp Group 2 | mAb 1D8 Biotinylated mAb 7A1 |
| Cat (<i>Felis domesticus</i>) Fel d 1 | mAb 6F9 Biotinylated mAb 3E4 mAb 1G9 |

| | | |
|---|--|--|
| | Cockroach (<i>Blattella germanica</i>) Bla g 1 | mAb 10A6 Rabbit polyclonal anti-CR antiserum |
| | Cockroach (<i>Blattella germanica</i>) Bla g 2 | mAb 7C11 Biotinylated mAb 8F4 |
| 5 | Aspergillus (<i>A. fumigatus</i>) Asp f 1 | mAb 4A6 Rabbit polyclonal anti-Asp f 1 |

10 The above-mentioned antibodies are available from Indoor Biotechnologies Ltd of 123 Deansgate, Manchester, M3 2BU, UK.

15 Allergen assays for detecting either mite Group 1 allergens or mite Group 2 allergens or both mite Group 1 and Group 2 allergens may be provided. Group 2 allergens are produced by at least two species of house dust mite and therefore detection indicates the presence of either of these species. There may be advantages to detecting both allergens. Group 2 allergens are less easily destroyed than Group 1 allergens and therefore
20 assessment of precisely which allergens were present would provide a better indication as to which chemical avoidance treatments are required.

25 Suitably the porous support material is a nitrocellulose membrane, such as those obtainable from Advanced Microdevices (Pvt) Ltd, 20-21 Industrial Area, Amblata Cantt, 13001 India, which may be held or cast onto a rigid backing support for example of a plastics material. Suitably the pore size of the membrane is between 5-15 μ ,
30 suitably about 8 μ and the membrane thickness is in the range of 75-125 μ , suitably about 95 μ . It may be cast onto a backing material such as mylar, and supported in a plastics support, for instance a HIPS plastic support. The backing material will suitably be of a thickness with
35 is similar to or slightly greater than that of the

membrane, for example 80-150 μ , suitably 105 μ . The support material will suitably be in elongate form such as a conventional dipstick arrangement wherein the membrane is between 20 and 40mm wide, suitably about 25mm. The
5 plastics support will necessarily be larger, and a typical example will have dimensions of 75 x 260mm with a thickness of 500 μ .

In a preferred embodiment, the porous support is
10 accommodated within a housing of impermeable material. A plurality of porous supports may be provided with such a housing. The housing may be arranged so that the porous membrane is accessible to liquid under test. In
15 addition, the housing may be provided with an opening or transparent section in the region of the detection zone to allow observation of any visible signal which develops.

Suitable labels are known in the art. For home use, a
20 direct label which provides a visible signal immediately on binding is preferred. Gold particles may comprise one such direct label. These particles become visible when concentrated in the detection zone. Gold particles, preferably of less than 50 nm in size may be attached to
25 the first binding reagent eg in the form of a monoclonal antibody using conventional techniques.

When using a direct particulate label, the presence of first binding reagent in the detection zone can be noted
30 by, in the case of gold, a red colouration. The deeper the colour, the higher the concentration of label in the detection zone, (although the colour will depend to a certain extent on the size of the detection zone).

35 The first and second binding reagents can be applied to the porous support using conventional technology. For example, they may be applied using a printing device

which applies biochemical reagents. A preferred configuration is to apply the second binding reagent as a thin line in the detection zone. This ensures that any labelled first reagent which binds in this area is concentrated as far as possible to provide a clear signal.

Preferably the assay is arranged so that a visible signal develops within a relatively short time period for example of less than 20 minutes, suitably within 5 to 15 minutes.

By providing a rapid assay which can specifically detect the presence of particular allergens in eg the home environment, patients suffering from allergic disease will be able to monitor their own exposure to those allergens and this should encourage compliance with avoidance procedures. Additionally, families with a genetic predisposition to asthma can monitor their environment and take action to avoid the onset of asthma in children of the family.

Furthermore, the assay is very specific for particular allergens such as are found in house dust and also highly sensitive. Detection limits of 100pg for the Der p 1 allergen, 50 to 100pg for the mite Group 2 allergens, 100pg for the Fel d 1 and 200pg for Bla g 2 allergen, are representative of levels which may be achieved. This is important when dealing with dust extracts where the concentration of allergens available to move through the porous support is very low.

The tests may be sold individually or in combination. A range of rapid test allergen assay devices for different allergens may be sold together as a kit to allow a complete allergen analysis of a particular environment to be carried out. The kits may comprise a series

individual assay devices as described above which can be tested either together or sequentially. Alternatively, a number of porous supports may be contained within a single housing which provides access to the liquid under test to all the supports simultaneously and wherein the detection zone of each support is visible.

Further each assay device may be designed to specifically detect the presence of a plurality of particular allergens which may be found in a sample eg of house dust. In which case, the device will comprise a pair of binding reagents specific for separate allergens of the plurality, each pair of binding reagents comprising a first labelled binding reagent (b) and a second binding reagent (c) as set out above.

The invention will now be described in detail by way of example only and with reference to the accompanying figures as earlier described. The invention is particularly exemplified with reference to dust samples. However the approach can be readily applied by those skilled in the art to other sources of allergens. Thus it may, for example, be applied to the detection of allergens in food eg nuts. In which case the food sample would be macerated and mixed with a liquid diluent and the liquid diluent processed as described above using first and second binding reagents appropriate for allergens suspected as being within the food sample.

Example 1

A support (1) comprising a 5mm nitrocellulose membrane shaped as a dipstick was prepared and a capture antibody comprising the antibody 5H8 at a concentration of 1 $\mu\text{g}/\text{cm}$ in phosphate buffered saline (PBS) p.H. 7.4 was printed onto the membrane in a line transversely across the membrane in detection zone (2). Purified detector antibody 4C1 was labelled with 40nm gold particles.

Detector antibody (4C1 dust mite) at a concentration of 5.2µg/ml was added to each member in a dilution series of solutions made from *Dermatophagoides pteronussinus* extract (10,000 AU/ml) containing 25µg/ml Der p 1 allergen and a control solution which contained no allergen extract.

An end (3) of a dipstick was immersed in each of the thus prepared solutions and the solution allowed to travel along the dipstick to the detection zone. Within a period of 5 to 10 minutes, a red line had appeared in the detection zone of all the dipsticks except those which had been treated with control solution.

Example 2

The process of Example 1 was repeated but instead of the Der p 1 allergen, extracts containing the following allergens were tested using the given combination of capture and detection antibody:

| Allergen Extract | Capture Antibody | Detection Antibody |
|------------------|------------------|---------------------------------|
| Der f 1 | 6A8 | Gold labelled 4C1 (5.2µg/ml) |
| Der p 2 | 1D8 | Gold labelled 7A1 (6.0µg/ml) |
| Fel d 1 | 6F9 | Gold labelled 3E4 (5.4µg/ml) |

Similar results to those found in Example 1 were detected.

Example 3

Example 2 was repeated, but instead of allergen extract, samples of house dust extract were used. House dust was collected from a number of different houses using a vacuum cleaning device with a dust collector attached.

5 The dust was sieved in order to remove large debris and a 100mg sample of the remainder was shaken in the presence of 2ml of buffered saline. Addition of detector antibody solution as set out in Example 2 was added to each sample and a dipstick added.

10 In all cases, red lines developed within 10 to 15 minutes. The depth of colour of the red line varied from one sample to another indicating the various concentrations of the different allergens in the different samples.

Example 4

15 An assay device was prepared as in Example 1 except the capture antibody was 6F9 at a concentration of $1\mu\text{g}/\text{cm}$ in PBS pH 7.4. The gold labelled detector antibody was 3E4 at a concentration of $5.4\mu\text{g}/\text{ml}$. House dust was collected from a number of different homes in Manchester, UK using a vacuum cleaning device with a dust collector attached.

20 The dust was sieved in order to remove large debris and a 100mg sample of the remainder was shaken in the presence of 2ml of buffered saline to provide the dust samples for assay. Detector antibody (3E4) at a concentration of $5.4\mu\text{g}/\text{ml}$ was added to each of a series of solutions comprising Felis domesticus extract allergen

25 extract(94/01, obtained from Indoor Biotechnologies Ltd, see above) containing 1.6 units/ml Fel d 1 and serially diluted to contain 80, 40, 20, 10, 5, 2.5 and 1 ng/ml Fel d 1 allergen and a control solution which contained no

30 allergen to provide the control samples for assay. An end (3) of the dipstick device carrying the Fel d 1 capture antibody was immersed in each of the control sample and dust sample solutions as described above and the

35 solutions allowed to travel along the dipsticks to the detection zone. Within a period of 10-15 minutes red lines had appeared in the detection zone of some of the dipsticks.

The results are shown in Fig. 2. Each of the dipsticks in the Fig.2 control curve series is for a different concentration of allergen starting at 80 ng/ml and going down to 1ng/ml as set out above. The assay sensitivity is approximately 2.5 ng/ml, equivalent to about 100pg per test (as determined by a quantitative plate-type enzyme immunoassay (EIA) to measure the allergen Fel d 1 in the dust samples).

10 Example 5

Assay devices as described in Example 4 were prepared and used. In this example the dust was collected from the air filters of vacuum cleaners used in US homes. The results are shown in Fig. 3. In most cases the dust sample contained Fel d 1 detectable by the dipstick assay device. The dust samples were separately assayed by EIA as stated in Example 4. Where the dipsticks in Fig. 3 are marked ▼, EIA results gave a dust sample Fel d 1 concentration of less than 1 mU/ml. Where the dipsticks in Fig. 3 are marked •, EIA results gave a dust sample Fel d 1 concentration of greater than 100 mU/ml.

Example 6

Assay devices as described in Example 4 were prepared and used. Bedding dust was collected from a number of different homes in São Paulo, Brazil using a vacuum cleaning device with a dust collector attached. The dust was sieved in order to remove large debris and a 100mg sample of the remainder was shaken in the presence of 2ml of buffered saline in order to provide dust samples for assay. Some of the homes had a cat in residence.

The results are shown in Fig. 4. In a number of cases, the bedding dust sample contained Fel d 1 detectable by the dipstick assay device. The dust samples were separately assayed by EIA as stated in Example 4. Where the dipsticks in Fig. 4 are marked ▼, EIA results gave a

dust sample Fel d 1 concentration of less than $1\mu\text{g/g}$. Where the dipsticks in Fig. 4 are marked •, EIA results gave a dust sample Fel d 1 concentration of less than or equal to $8\mu\text{g/g}$. Where the dipsticks in Fig. 4 are marked 'C', the samples came from homes with a cat in residence. The dipsticks detected Fel d 1 in: all dust samples from homes with a cat in residence; all dust samples containing up to $8\mu\text{g/g}$ Fel d 1 as determined by EIA; and in some dust samples containing up to $1\mu\text{g/g}$ Fel d 1 as determined by EIA.

Example 7

An assay device was prepared as in Example 1 except the capture antibody was 1D8 at a concentration of $1\mu\text{g/cm}$ in PBS pH 7.4. The gold labelled detector antibody was 7A1.

House dust was collected separately from the kitchen floor, the bedroom floor and bedding in a number of different homes in São Paulo, Brazil using a vacuum cleaning device with a dust collector attached. The dust was sieved in order to remove large debris and a 100mg sample of the remainder was shaken in the presence of 2 ml of buffered saline (dust samples).

Detector antibody 7A1 at a concentration of $6\mu\text{g/ml}$ was added to a series of solutions comprising *D. pteronyssinus* allergen extract (92/02, obtainable from Indoor Biotechnologies Ltd, see above) containing 5000ng/ml Der p 2 and serially diluted to contain 250, 125, 63, 32, 16, 8, 4, 2 and 1 ng/ml Der p 2 allergen and a control solution which contained no allergen. These solutions comprise the Der p 2 control samples.

Detector antibody 7A1 at a concentration of $6\mu\text{g/ml}$ was added to a series of solutions comprising *D. farinae* allergen extract (92/02, obtainable from Indoor Biotechnologies Ltd, see above) containing

250, 125, 63, 32, 16, 8, 4, 2 and 1 ng/ml Der f 2 allergen and a control solution which contained no allergen. These solutions comprise the Der f 2 control samples.

5 An end (3) of the dipstick carrying the 1D8 capture antibody which is directed against both Der p 2 and Der f 2 allergens, was immersed in each of the samples (dust: kitchen; bedroom floor and bedding; Der p2 control samples; and Der f 2 control samples).

10

The results are shown in Figs 5, 6, 7 and 8. Fig. 5 shows that the dipstick device has a sensitivity of 1-2 ng/ml, equivalent to about 50 to 100pg per test. The dipstick device was also able to detect the mite Group 2 allergens (Der p 2 and Der f 2) allergen in house dust samples obtained from the kitchen floors, bedroom floors and bedding.

15

The dust samples were separately assayed by EIA as stated in Example 4. Where the dipsticks in Fig. 6, 7 and 8 are marked ▼, EIA results gave dust sample mite Group 2 (Der p 2 and Der f 2 allergens) concentrations of less than 1µg/g. Even at this low level of allergen concentration the dipstick was able to detect the presence of the allergens in samples from kitchen floors, bedroom floors and bedding. Where the dipsticks in Fig. 6 are marked •, EIA results gave a dust sample mite Group 2 allergens (Der p 2 and Der f 2) concentrations of greater than 10µg/g.

20

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CLAIMS:

1. A method for testing for the presence of a particular allergen which comprises:

- (a) obtaining a substantially unpurified sample suspected of containing an allergen;
- (b) if a non-liquid sample mixing it with a liquid diluent and if a liquid sample optionally diluting it with liquid diluent to produce a liquid sample being a solution and/or suspension;
- (c) contacting the liquid sample or a portion thereof with a labelled first binding reagent for a said allergen, the reagent being freely movable through a porous support of an assay device;
- (d) allowing said first binding reagent whether free or complexed to said allergen to move along a said porous support to meet a second binding reagent for said allergen immobilised at a detection zone in the support, wherein said first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a said binding reagent is specific for an epitope characteristic of said allergen; and
- (e) examining for labelled binding reagent at said detection zone, the presence of labelled binding reagent at said detection zone being indicative of the absence/presence of allergen in the sample, depending on whether said first and second binding reagents are with respect to each other, competitive or non-competitive for said allergen.

2. A method according to claim 1 wherein the step of

contacting the liquid sample or a portion thereof with said labelled first binding reagent occurs in the porous support.

5 3. A method according to claim 1 wherein the step of contacting the liquid sample or a portion thereof with said labelled first binding reagent occurs in a vessel external to the support.

10 4. A method according to any one of claims 1 to 3 wherein said first and second binding reagents are with respect to each other non-competitive for said allergen.

15 5. A method according to any one of claims 1 to 3 wherein said first and second binding reagents are with respect to each other competitive for said allergen.

20 6. A method according to any one of claims 1 to 5 wherein a said binding reagent comprises a substance with an antibody binding domain specific for said allergen.

7. A method according to claim 6 wherein the antibody binding domain derives from a monoclonal antibody.

25 8. A method according to any one of claims 1 to 7 wherein a said binding reagent comprises a monoclonal antibody or a fragment thereof.

30 9. A method according to any one of claims 1 to 8 wherein said first and second binding reagents both comprise substances with a monoclonal antibody binding domain specific for said allergen.

35 10. A method according to claim 9 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment.

11. A method according to claim 10 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment, which antibody or antibody fragment has an antibody binding domain specific for a said epitope characteristic of said allergen.
12. A method according to any one of claims 1 to 11 wherein the allergen is one of cat, cockroach, aspergillus or dust mite.
13. A method according to claim 12 for testing for the presence of mite Group 1 allergens, or mite Group 2 allergens or both mite Group 1/Group 2 allergens.
14. A method according to any one of claims 1 to 13 wherein the porous support comprises a nitrocellulose membrane.
15. A method according to claim 14 wherein the porous support is accommodated within a housing.
16. A method according to any one of claims 1 to 15 wherein the labelled first binding reagent comprises a gold label.
17. A method according to any one of claims 1 to 16 wherein the absence/presence of allergen in the sample is determinable within 20 minutes of contacting the liquid dust sample with said labelled first binding reagent.
18. A method according to claim 17 wherein the absence/presence of allergen in the sample is determinable within 15 minutes.
19. A method according to claim 17 wherein the absence/presence of allergen in the sample is

27

determinable within 10 minutes.

20. A method according to claim 17 wherein the
absence/presence of allergen in the sample is
5 determinable within 5 minutes.

21. A method according to any one of claims 1 to 20 for
testing for the presence of a plurality of particular
allergens which may be found in the sample.

10 22. A method according to claim 21 which utilises a
plurality of porous supports, each support being for
testing for the presence of one particular allergen of
the plurality of particular allergens.

15 23. A method according to claim 21 which utilises a
single porous support for testing for the presence of
more than one particular allergen of the plurality of
particular allergens.

20 24. A method according to any one of claims 21 to 23
which comprises a plurality of pairs of first and second
binding reagents, each pair being for testing for the
presence of one particular allergen of the plurality of
25 particular allergens.

25. A method according to claim 24 wherein a common
first or second binding reagent is employed for testing
for the presence of more than one particular allergen of
30 the plurality of particular allergens.

26. A method according to any one of the preceding
claims wherein the sample is selected from dust, water,
air, a foodstuff or a drink.

35 27. A method according to claim 26 wherein the sample is
a dust sample.

28. A method according to claim 26 or claim 27 wherein the sample is a house dust sample.

5 29. A method as described herein with reference to one or more of examples 1 to 7.

10 30. An assay system for testing for the presence of a particular allergen which may be found in a substantially unpurified sample suspected of containing an allergen which comprises (a) a porous support which bears a solution and/or suspension of the sample, (b) a labelled first binding reagent for a said allergen, the reagent being freely movable through a porous support of an assay device, (c) a second binding reagent for said allergen
15 which second reagent is immobilised at a detection zone in the support and wherein the first and second binding reagents are with respect to each other, either competitive or non-competitive for said allergen and a said binding reagent is specific for an epitope
20 characteristic of said allergen.

25 31. An assay system according to claim 30 wherein said labelled first binding reagent is in a vessel external to the support.

32. An assay system according to claim 30 wherein said labelled first binding reagent is in the porous support.

30 33. An assay system according to any one of claims 30 to 32 wherein said first and second binding reagents are with respect to each other non-competitive for said allergen.

35 34. An assay system according to any one of claims 30 to 32 wherein said first and second binding reagents are with respect to each other competitive for said allergen.

35. An assay system according to any one of claims 30 to 34 wherein a said binding reagent comprises a substance with an antibody binding domain specific for said allergen.

5

36. An assay system according to claim 35 wherein the antibody binding domain derives from a monoclonal antibody.

10

37. An assay system according to any one of claims 30 to 36 wherein a said binding reagent comprises a monoclonal antibody or a fragment thereof.

15

38. An assay system according to any one of claims 30 to 37 wherein said first and second binding reagents both comprise substances with a monoclonal antibody binding domain specific for said allergen.

20

39. An assay system according to claim 38 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment.

25

40. An assay system according to claim 39 wherein said first and second binding reagents both comprise either a monoclonal antibody or a monoclonal antibody fragment, which antibody or antibody fragment has an antibody binding domain specific for a said epitope characteristic of said allergen.

30

41. An assay system according to any one of claims 30 to 40 wherein the allergen is one of cat, cockroach, aspergillus or dust mite.

35

42. An assay system according to claim 41 for testing for the presence of mite Group 1 allergens, or mite Group 2 allergens or both mite Group 1/Group 2 allergens.

43. An assay system according to any one of claims 30 to 42 wherein the porous support comprises a nitrocellulose membrane.

5 44. An assay system according to claim 43 wherein the porous support is accommodated within a housing.

45. An assay system according to any one of claims 30 to 44 wherein the labelled first binding reagent comprises a
10 gold label.

46. An assay system according to any one of claims 30 to 45 wherein the absence/presence of allergen in the dust sample is determinable within 20 minutes of contracting
15 the liquid sample with said labelled first binding reagent.

47. An assay system according to claim 46 wherein the absence/presence of allergen in the sample is
20 determinable within 15 minutes.

48. An assay system according to claim 46 wherein the absence/presence of allergen in the sample is determinable within 10 minutes.
25

49. An assay system according to claim 46 wherein the absence/presence of allergen in the sample is determinable within 5 minutes.

30 50. An assay system according to any one of claims 30 to 49 for testing for the presence of a plurality of particular allergens which may be found in the sample.

35 51. An assay system according to claim 50 which utilises a plurality of porous supports, each support being for testing for the presence of one particular allergen of the plurality of particular allergens.

52. An assay system according to claim 50 which utilises a single porous support for testing for the presence of more than one particular allergen of the plurality of particular allergens.

5

53. An assay system according to any one of claims 50 to 52 which comprises a plurality of pairs of first and second binding reagents, each pair being for testing for the presence of one particular allergen of the plurality of particular allergens.

10

54. An assay system according to claim 53 wherein a common first or second binding reagent is employed for testing for the presence of more than one particular allergen of the plurality of particular allergens.

15

55. An assay system according to any one of claims 30 to 54 wherein the sample is selected from dust, water, air, a foodstuff or a drink.

20

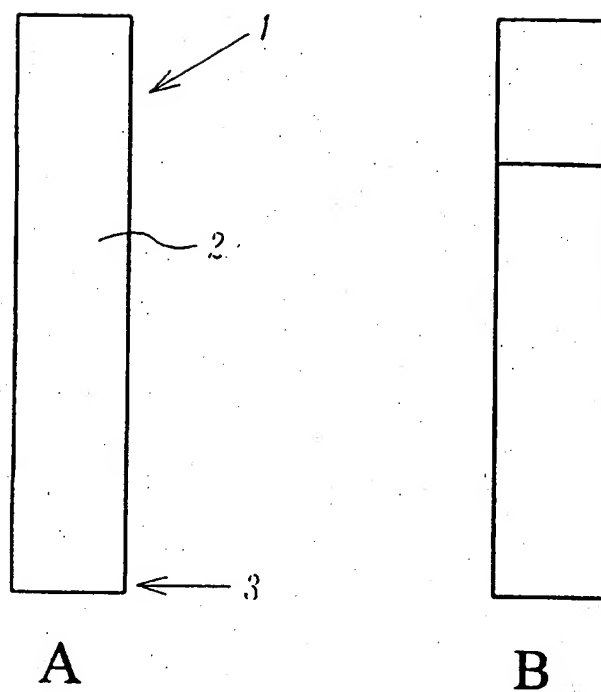
56. An assay according to claim 55 wherein the sample is a dust sample.

57. An assay according to claim 55 or claim 56 wherein the sample is a house dust sample.

25

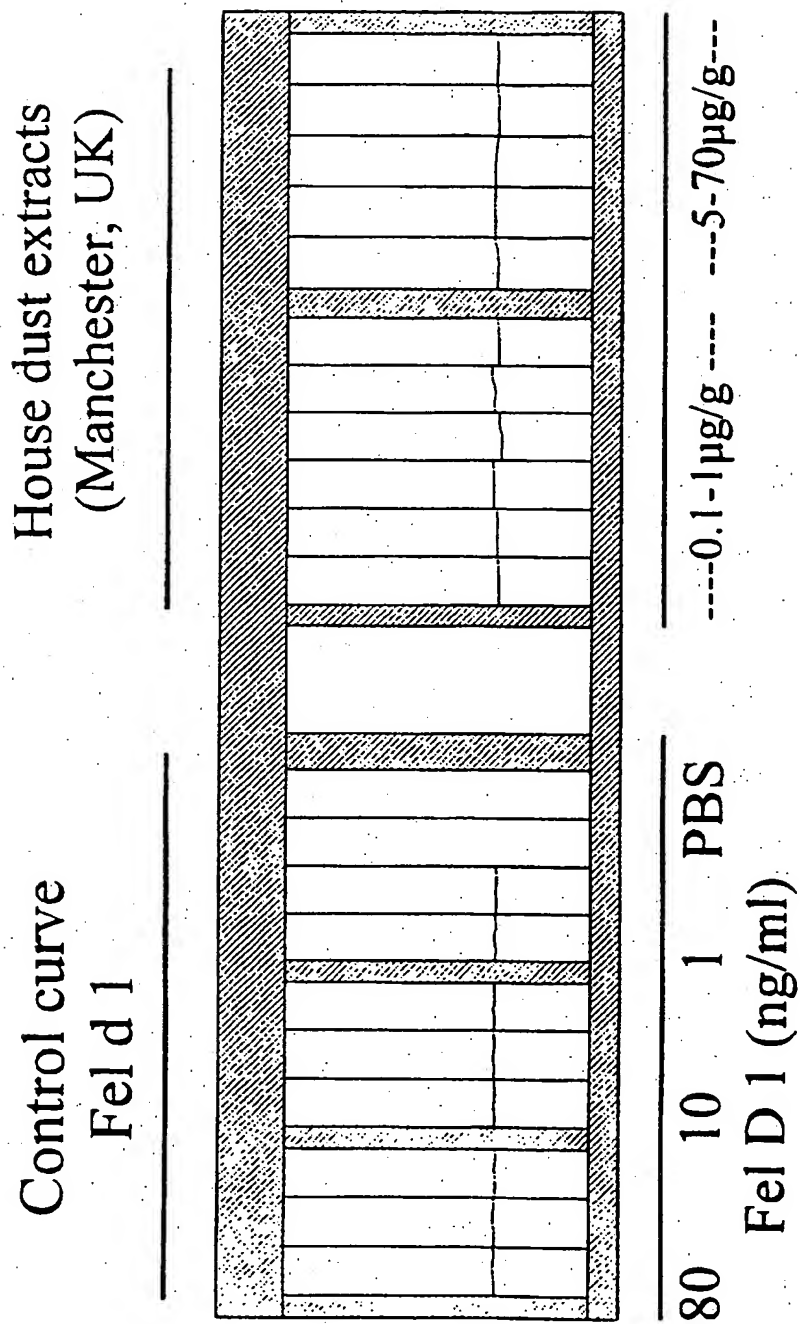
58. An assay system as described herein with reference to one or more of examples 1 to 7.

1/8

Fig. 1

2/8

Fig. 2

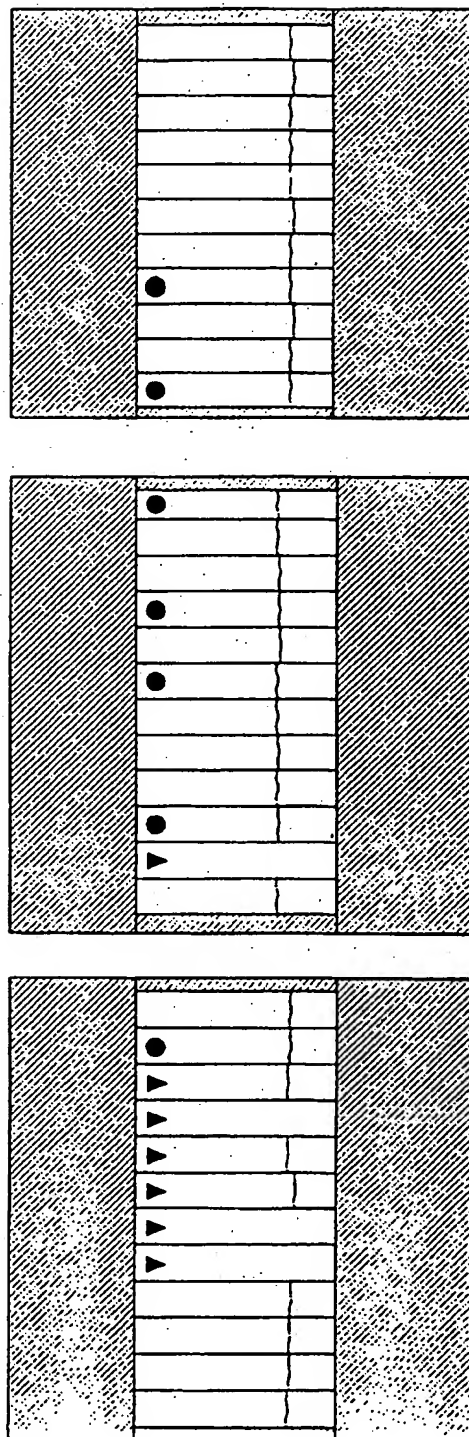


Sensitivity: 2.5ng/ml, equivalent to ~100pg per test

3/8

Fig. 3

Detection of Feld 1 in Air Filter Samples
from U.S. homes (n=39)

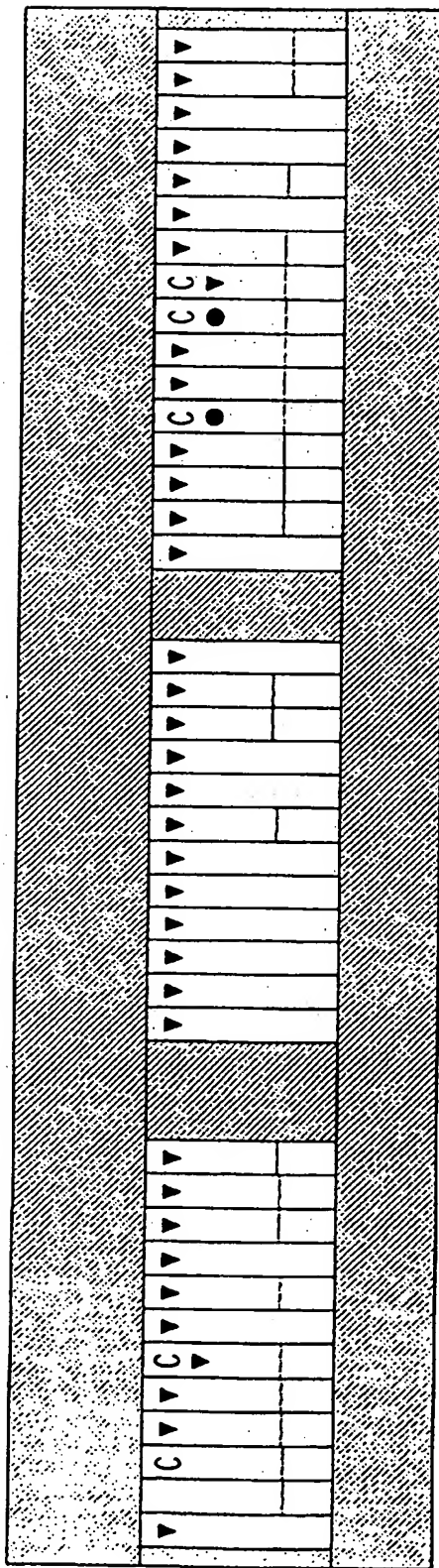


Key: ▼ = < 1mU/ml; ● = ≥ 100mU/ml

4/8

Fig. 4

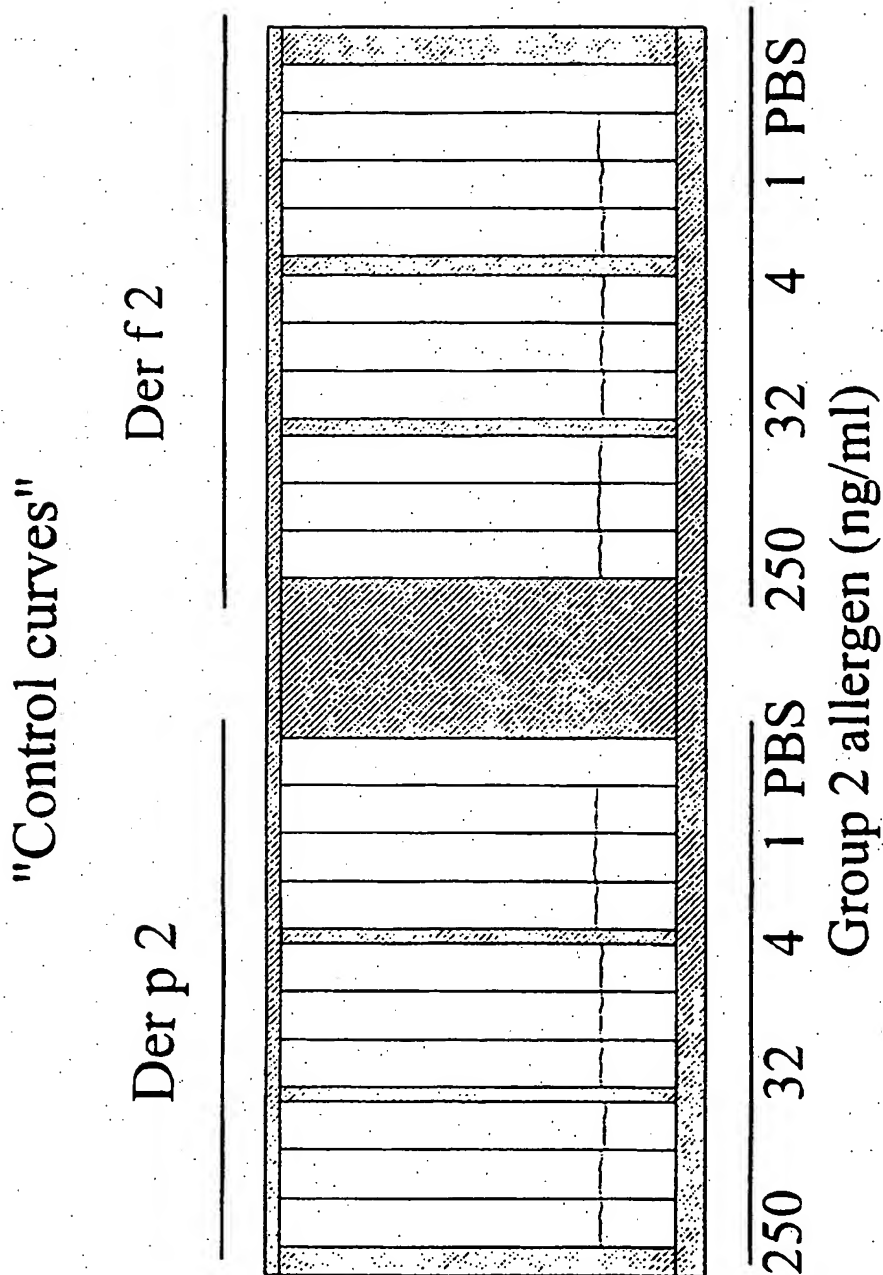
**Fel d 1 in House Dust Extracts from
São Paulo, Brazil (n=40)**



C = homes with cat or cat in yard; ▼ = <1 µg/g Fel d 1; ● = ≥8 µg/g

5/8

Fig. 5

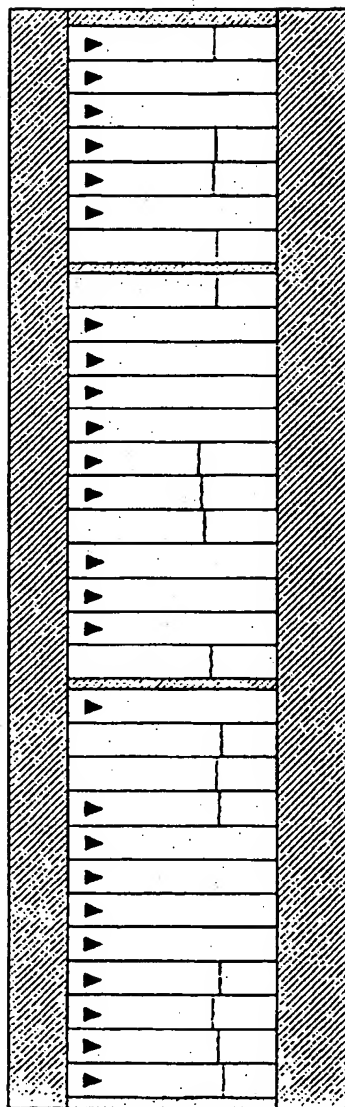


Sensitivity: 1-2ng/ml, equivalent to ~50 - 100pg per test

6/8

Fig. 6

Mite Group 2 allergen in House Dust Extracts from São Paulo, Brazil

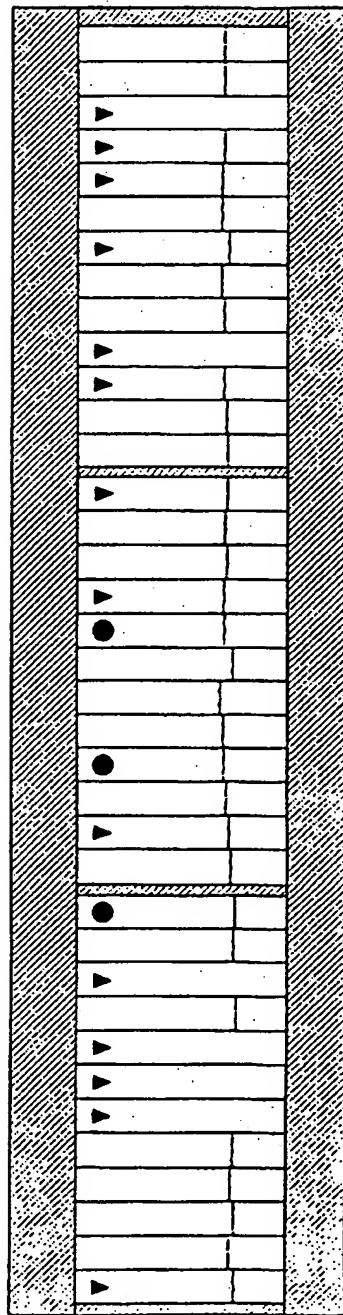


Kitchen dust (n=31); ▼ =<1 µg/g Group 2; ● =>10 µg/g

7/8

Mite Group 2 allergen in House Dust Extracts from São Paulo, Brazil

Fig. 7

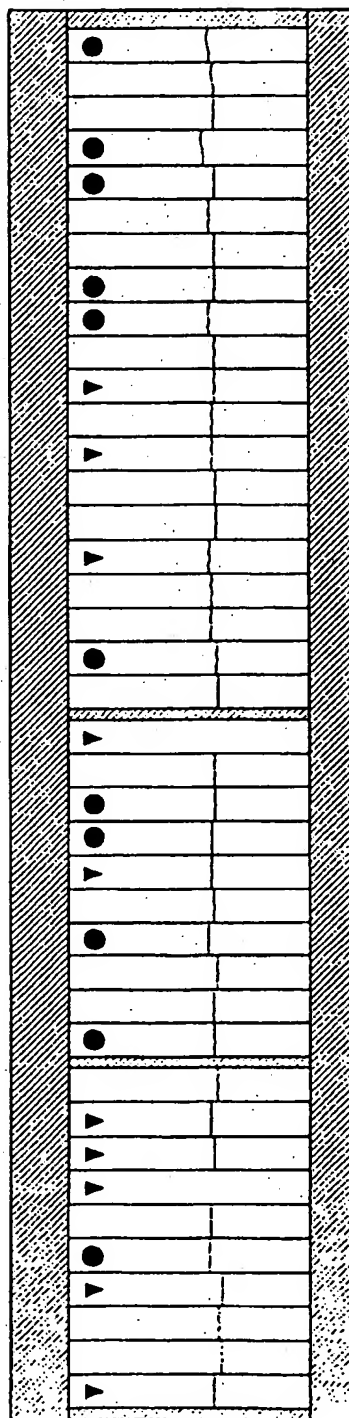


Bedroom floor samples (n=37); ▼ = <1 µg/g Group 2; ● = ≥10 µg/g

8/8

Fig. 8

Mite Group 2 allergen in House Dust Extracts from São Paulo, Brazil



Bedding dust samples (n=40); ▼ =<1 µg/g Group 2; ● = ≥10 µg/g

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 97/00223

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/558 G01N33/543 G01N33/577 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|---|-----------------------|
| X | WO 94 29696 A (QUIDEL CORPORATION) 22 December 1994 see the whole document --- | 1-58 |
| Y | US 4 727 037 A (D. B. RING.) 23 February 1988 see the whole document --- | 1-58 |
| Y | WO 89 00508 A (A.-M. FRANCOEUR.) 26 January 1989 see claims 24-29 --- | 1-58 |
| Y | EP 0 615 128 A (LABORATORIO FARMACEUTICO LOFARMA S.R.L.) 14 September 1994 cited in the application see the whole document --- | 1-58 |
| -/- | | |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

5 June 1997

Date of mailing of the international search report

16.06.97

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INTERNATIONAL SEARCH REPORT

International Application No.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 97/00223

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